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Do differences in developmental mode shape the potential for local adaptation?

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Title: Do differences in developmental mode shape the potential for local adaptation?

Running Title: Is dispersal the key to local adaptation?

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Abstract

Future climate change is leading to the redistribution of life on Earth as species struggle to cope with rising temperatures. Local adaptation allows species to become locally optimised and persist despite environmental selection, but the extent to which this occurs in nature may be limited by dispersal and gene flow. Congeneric marine gastropod species (*Littorina littorea* and *L. saxatilis*) with markedly different developmental modes were collected from across a latitudinal thermal gradient to explore the prevalence of local adaptation to temperature. The acute response of metabolic rate (using oxygen consumption as a proxy) to up-ramping and down-ramping temperature regimes between 6-36ºC was quantified for five populations of each species. The highly dispersive *L. littorea* exhibited minimal evidence of local adaptation to the thermal gradient, with no change in thermal optimum (Topt) or thermal breadth (Tbr) and a decline in maximal performance (μmax) with increasing latitude. In contrast, the direct developing *L. saxatilis* displayed evidence of local optimisation, although these varied

idiosyncratically with latitude, suggesting a suite of selective pressures may be involved in shaping thermal physiology in this relatively sedentary species. Our results show that the biogeography of thermal traits can differ significantly between related species, and show that inter-population differences in thermal performance do not necessarily follow simple patterns which may be predicted based on latitudinal changes in environmental temperatures. Further research is clearly required to better understand the mechanisms that can lead to the emergence of local adaptation in marine systems and allow improved predictions of species redistribution in response to climate change.

Key Words: thermal tolerance; metabolic rate; gene flow; divergent natural selection; adaptive differentiation; marine invertebrate; species distribution; climate change.

Introduction

The redistribution of life on Earth is emerging as one of the most profound impacts of anthropogenic climate change on global biodiversity, with temperature thought to be the major driver in most systems (Sunday et al., 2012; Pecl et al., 2017). Although posing a risk to the fitness and survival of many species, projected temperature rises are expected to most severely impact ectotherms, due to the direct link between environmental and body temperatures in these animals (Angilletta, 2009). Through its influence on metabolism, physiological processes, performance and ultimately fitness, temperature places rigid constraints on where an ectotherm can live (Pörtner, 2002; Somero, 2005; Angilletta, 2009; Hall and Thatje, 2011; Gannon et al., 2014). As a consequence, the geographical distributions of such species are expected to be highly responsive to future temperature rise (Deutsch et al., 2008; Cheung et al., 2009; Sunday et al., 2012).

Our understanding of how temperature affects molecular, physiological and behavioural responses in ectotherms has grown rapidly in recent years, providing insights into thermal niches and tolerance windows across a range of taxa and environments (Kassahn et al., 2009; Calosi et al., 2010; Pörtner, 2010; Du and Shine, 2015; Shulte, 2015; Arbona et al., 2016; Rangel and Johnson, 2018). Surprisingly, few studies have extrapolated these findings to explore the role of temperature in driving broad-scale biogeography (Kearney and Porter, 2009) but most commonly, 'climate envelope models' are used to link a species' geographic distribution with its environmental requirements (or climate envelope) (Thomas et al. 2004). These models can serve as useful, if crude, range-shift predictors for multiple taxa over broad geographic scales (Jeschke and Strayer, 2008). They can, however, struggle to predict biogeographical changes at finer spatial and biotic resolution due to the relative simplicity of the models (Gaston, 2003; Pearson and Dawson, 2003; Sanford and Bertness, 2009; Valladares et al., 2014), and/or failure to recognise important structural differences such as thermal niche diversity within species (Hoffmann et al., 2003; Fangue et al., 2006; Kuo and Sanford, 2009; Storch et al., 2009); variation which in the past was often considered a superficial artefact of acclimation to local environmental conditions by highly plastic phenotypes (Bennett and Beitinger, 1997; Schaefer and Ryan, 2006; Terblanche et al., 2006).

There may be an underlying genetic basis (Sørensen et al., 2001; Conover et al., 2006; Kuo and Sanford, 2009) to this variability, with possible mechanisms including environmental filtering and/or genetic drift leading to genetic differentiation of thermal traits (Palumbi, 1994; Sommer et al., 2014), or phenotypic plasticity (Alberto et al. 2013; Chuine & Beaubien 2001) allowing a species to adapt and/or evolve to changing conditions (Valladares et al. 2014). Local adaptation (adaptive genetic changes) could also explain spatial differences in performance as populations become 'locally optimised' to their environment through natural selection (Kawecki and Ebert,

2004). Optimisation may include a narrowing of thermal niche width, in exchange for greater maximal performance and thermal performance optima (Angilletta, 2009). Optimisation, however, could be achieved through plasticity (e.g. Chevin et al. 2010), adaptation or a combination of both making differentiating the effects of plasticity and genetic differentiation challenging. To do so requires relatively complex experiments to determine whether quantitative trait differences among populations disappear when subsequent generations are reared under the same conditions (i.e. using reciprocal transplants; Martin et al. 2007, Calosi et al. 2008).

Current theory suggests that the scale over which adaptive differentiation occurs directly reflects the balance between gene flow and the grain of the selective gradient (Sanford and Kelly, 2011). To date, evidence of local adaptation has largely come from studies of terrestrial and freshwater taxa (e.g. Castañeda et al., 2004; Kawecki and Ebert, 2004; Narum et al., 2013; Gao et al. 2018); habitats often characterised by fine-grained selective gradients and physical barriers to movement which can restrict the dispersal and gene flow capabilities of their inhabitants (Hereford, 2009). In contrast, few studies have addressed this phenomenon within the marine environment (Conover, 1998; Sotka, 2005). Research in this area has been hindered by the common perception that marine species are typically comprised of demographically open populations, interconnected by high levels of gene flow (Caley et al., 1996; Grosberg and Cunningham, 2001). A large proportion of marine species possess a planktonic dispersal phase with potential for gene flow over many hundreds of kilometres if unimpeded by isolating barriers (Scheltema, 1986; Shanks, 2009). Combined with comparatively coarser environmental gradients in the ocean, these factors have led many to assume that the diversifying effects of selection would be overpowered by the homogenising effects of gene flow in most marine species. However, many marine populations are increasingly considered less well connected than originally thought (e.g. Cowen et al., 2000; Palumbi, 2004), and there is evidence that genetic

differentiation at specific loci may still arise between 'well-mixed' populations (Schmidt and Rand, 2001), increasing the likelihood of widespread local adaptation within marine environments.

Increasingly, there is evidence to suggest that variation in temperature has led to adaptive divergence in physiological traits among marine populations distributed across a variety of scales (Imsland et al., 2001; D'Croz and Maté, 2004; Hays, 2007), notably latitudinal temperature gradients (Lonsdale and Levington, 1985; Sokolova and Pörtner, 2001; Kuo and Sanford, 2009). Many early examples of local adaptation were derived from species possessing low dispersal capabilities (e.g. direct developers), although surprisingly, the majority of evidence has been demonstrated in planktotrophic developers with high dispersal capacities (Sanford and Kelly, 2011; Burford et al., 2014), suggesting that gene flow may exert less influence in this process than previously assumed. However, our ability to elucidate the dynamics of local adaptation to temperature in oceanic environments is limited by the experimental design of most studies on this topic, which typically compare a low number of highly distant populations belonging to a single species (Sanford and Kelly, 2011). As such, there remains a need to characterise the geographic patterns and scale of adaptive differentiation in thermal traits among marine populations, with an emphasis on spatially intensive sampling and comparisons of closely related species with different life history traits. Disentangling the effects of local adaptation and phenotypic plasticity in producing localised differences in thermal traits is also critical, as the implications of each phenomena for species vulnerability and persistence under climate change are predicted to be very different (Sanford and Kelly, 2011; Valladares et al., 2014; Forsman, 2015).

This study aimed to assess the extent to which the scale of gene flow between marine populations influences the strength of adaptive differentiation in thermal traits across a latitudinal temperature gradient. Two intertidal congeners, *Littorina littorea* and *L. saxatilis*, characterised by high and low dispersal capabilities respectively, are compared using thermal performance curves (TPCs) to assess acute thermal sensitivity of metabolism among populations toward the centre of their range (Figure 1). At species range edges, distribution limits are argued to be imposed by environmental constraints resulting in reduced individual fitness in comparison to individuals from the centre of the distribution (Kawecki 2008). Geographically peripheral populations do not always occur in unfavourable habitats (Jump & Woodward 2003; Granado-Yela et al. 2013), with fitness declines at environmental margins dependent on the extent of local adaptation, phenotypic plasticity, the steepness of environmental gradients, and gene flow, amongst other factors (Valladares et al. 2014; Kawecki 2008; Lenoir & Svenning 2013). Nevertheless, identifying differences in thermal sensitivity toward the centre of species distributional ranges would suggest marked effects of local adaptation. Intertidal species are considered most at risk under future climate scenarios due to their narrow thermal safety margins and low capacity for acclimation (Stillman and Somero, 2000; Stillman, 2003) and, therefore, comparisons of these congeners presents a valuable opportunity to assess the extent to which gene flow influences adaptive differentiation in relative isolation from other life history traits.

It is predicted that the differences in dispersal capacity between the two species will result in differing levels of adaptation to the thermal gradient, with *L. saxatilis* (low disperser) showing greater inter-population differentiation in its response to the temperature treatments compared to the highly dispersive *L. littorea*. Based on thermal adaptation theory, where differentiation is evident, thermal optima and thermal maxima should be expected to decrease and increase with increasing latitude, respectively (due to a lowering of mean temperature and an increase in

seasonal temperature variability). Furthermore, maximal performance should show a compensatory decline with increasing thermal breadth, as would be expected under the specialist-generalist trade-off theory (Figure 2).

Methodology

Model Species

Littorina littorea and *L. saxatilis* are benthic gastropods native to the North Atlantic and exhibit broad latitudinal distributions along coastlines from Iberia to Scandinavia (Figure 1), being common on rocky shores over most of this range. The distribution of *L. littorea* is bounded by populations in Northern Spain (42°N) and the White Sea (70°N) (Bequaert, 1943; Fretter and Graham, 1976), whereas *L. saxatilis* has a greater latitudinal extent, occurring as far as Gibraltar (36°N) in the South and Novaya Zemlya (74°N) in the North (Davis, 1971; Gofas, 1975).

These species possess different developmental modes, which strongly influence their capacity for dispersal. *Littorina littorea* produces planktotrophic larvae (with an early lecithotrophic phase) that remain planktic for between 4-8 weeks, potentially facilitating dispersal over 10 -100 km (Fretter and Graham, 1976; Johannesson, 2008). In contrast, *L. saxatilis* is a direct developer, brooding embryos until they hatch as post-metamorphic crawling juveniles with a consequently low dispersal capability, likely in the order of metres (Fretter and Graham, 1976). Phylogenetic data indicates genetic structure among *L. saxatilis* populations congruent with restricted dispersal (Doellman et al. 2011); structure not seen in *L. littorea*, which show low levels of differentiation over thousands of kilometres (Johannesson, 1992).

Specimen Collection and Acclimation

174 Five populations of *L. littorea* and *L. saxatilis* were sampled from the western coast of France
175 and southern United Kingdom (Figure 1) covering ~ 4° of latitude and a distance of 475 km.
176 Locations are characterised by different means, extremes and variability in sea surface
177 temperatures (Figure 3). All sites are located on the Atlantic seaboard and typically experience
178 high wave fetch (www.EMODnet-physics.eu) and thus well-flushed, are fully marine in terms of
179 salinity (~34; www.euro-argo.eu), well-oxygenated (>6mg/L, www.oap.ospar.org), have similar
180 pH (IPCC 2017), and are characterised by high levels of primary production (e.g. Chl-a;
181 www.oap.ospar.org).
182
183 Sampling of populations along the western coast of France was conducted between the 26th and
184 28th of July 2017. Fifty adult individuals of each species were haphazardly collected at each site
185 from the zone of their uppermost vertical distribution on natural rocky shorelines. Live snails
186 were transported in ventilated cool boxes containing seawater (changed daily) and fresh *Ulva*
187 *lactuca* as a food source. Mortality during transport was minimal (<5% of individuals).
188 Specimens were transported to the Marine Biology and Ecology Research Centre at Plymouth
189 University (Plymouth, UK) within 72 h of collection.
190
191 On return to the laboratory, live snails were immediately transferred to 8 L plastic aquaria
192 (maximum of 100 individuals per aquarium) filled to two-thirds of their maximum volume with
193 aerated seawater (pH 8.01, salinity 35, 15°C). Aquaria were housed in a temperature-controlled
194 room maintained at 15°C (12:12 h light/dark regime). Snails were fed *ad libitum* on fresh *U.*
195 *lactuca*, and full water changes were undertaken twice weekly to prevent the accumulation of
196 ammonia and ensure stable salinity and pH conditions.
197

198 All populations were maintained under these conditions for an acclimation period lasting a
199 minimum of 14 weeks. Based on a survey of comparable studies performed on littorinids, this
200 acclimation length was deemed sufficient for minimising potential differences in metabolic
201 physiology between populations attributable to their recent acclimatisation history in the field
202 (Hawkins, 1995; Sokolova and Pörtner, 2001, 2003; Calosi et al., 2017). Mortality was observed
203 to be low for all populations during the acclimation period (<5% of individuals).
204
205 Thermal Ramping Assays
206 Following acclimation, thermal ramping assays were performed to assess the thermal
207 dependence of aerobic metabolism in *L. littorea* and *L. saxatilis* from different populations.
208 Mass-specific aquatic oxygen consumption ($\dot{M}O_2$) was used as a proxy for routine metabolic rate
209 (RMR) (McMahon, 1988), defined as standard metabolic rate plus any extra metabolism due to
210 spontaneous activity and stress (Arnott et al., 2006).
211
212 Respiration measurements were undertaken in water, as typically, this is when Littorina are most
213 active and have reduced tendency toward closing their operculum; a response undertaken to
214 reduce desiccation risk in air but which limits re-oxygenation (e.g. McMahon 1990 and
215 references therein). The aquatic oxygen consumption of 120 individuals (~ 12 per population per
216 species) was measured at different temperatures between 6°C and 36°C using closed-system
217 respirometry. These temperatures reflect the broad range of aquatic and aerial temperatures
218 experienced over acute and chronic timescales by the different populations sampled (Figure 3).
219 Over the course of each experimental run, individuals were sequentially exposed to down-ramp
220 (15 ↠ 12 ↠ 9 ↠ 6°C) or up-ramp (15 ↠ 20 ↠ 25 ↠ 30 ↠ 33 ↠ 36°C) scenarios. Each
221 individual was exposed to one thermal ramping treatment only (i.e. up-ramp or down-ramp
222 scenario), and both ramping treatments started at the acclimation temperature, leading to twice as

223 many trials at 15°C than for any other temperature. Oxygen consumption at each temperature
224 was measured for individual snails housed in glass respiration chambers using a microfiber optic
225 oxygen sensor (PreSens Fibrox 4, Germany; www.presens.de). Due to high levels of variation in
226 snail size within and between populations and species, three different chamber volumes were
227 used for snails of different size classes (subdivided based on columellar length): 7 ml (<12 mm),
228 18 ml (12-18 mm), and 35 ml (>18 mm).
229
230 Two identical experimental systems were operated simultaneously during each experimental run,
231 each accommodating eight respiration chambers: two controls and six containing live animals.
232 Individuals from different populations and species were randomly assigned to different
233 experimental runs to account for random variability in experimental conditions between runs.
234 Respiration chambers were immersed in a temperature-controlled water bath (capacity 8 L) filled
235 with 2 μm filtered and autoclaved seawater (pH 8.01, salinity 35) oxygenated to at least 90%
236 saturation. Water baths were heated and cooled externally using a VersaCool™ refrigerated
237 circulating bath (ThermoScientific™; www.thermofisher.com) which circulated heated or cooled
238 effluent (ethanol glycol) through PVC piping affixed to the perimeter of the water bath.
239 Magnetic stir bars (300 rpm) were used to maintain constant water circulation within the baths,
240 keeping seawater aerated and ensuring a uniform water temperature. Prior to oxygen
241 consumption measurements, snails were fasted for 48h to minimise levels of aerobic metabolism
242 associated with digestion and the production of faeces during the experiment. Shells were treated
243 with 95% ethanol and gently scraped to remove any biofilm or associated epibiota.
244
245 All thermal assay experiments began from the acclimation temperature of 15°C and were started
246 at the same time of day in order to minimise the effects of the circadian rhythm on the aerobic
247 respiration rates of snails (Shirley and Findley, 1978). Upon transfer into the respiration

chambers, animals were given 1 h to recover from the stress of handling and to acclimate to experimental conditions. The temperature of the water bath was then raised or lowered to the desired experimental temperature by 1°C every ten minutes; a rate comparable to the rapid changes in aerial and water temperature experienced within the intertidal environment on a daily basis (Stenseng, 2005). Respiration chambers remained open during the initial acclimation period and between recording periods to maintain conditions of normoxia and eliminate any potential temperature lag between the water temperature inside the chamber and the water bath.

Once the desired experimental temperature was reached and allowed to stabilise, chambers were sealed and the water temperature held constant (± 0.25°C). Stir bars located beneath a perforated barrier in the base of each chamber circulated water (300 rpm) to prevent the formation of localised oxygen gradients. Measurements commenced 20 minutes after the chambers were sealed to allow time for the system to equilibrate and the animals to acclimate to the minor differences in flow conditions within the chamber. The dissolved oxygen concentration of the water within the respiration chamber (measured in μmol O_2 L^{-1}) was recorded and every five minutes thereafter for a total of 25 min, or until dissolved oxygen content dropped below 60% of normoxic values. This value was deemed appropriate as pre-experimental trials and later analysis of results showed the decline in oxygen content with time to be linear throughout duration of each recording period. Control chambers contained no animals and were used to correct for any background microbial oxygen consumption or drift associated with the oxygen probe. The activity levels and behaviour of each snail were also recorded throughout the experiment. At the end of each recording period, respiration chambers were reopened and the water bath temperature increased or decreased to the next experimental temperature at the aforementioned rate. This protocol was repeated until the full range of experimental temperatures had been tested.

Following the completion of an experimental run, snails were removed from their respiration chambers and whole body mass (g) and volume (ml) (determined by water displacement) were recorded.

Calculation of $\dot{V}O_2$

Mass-specific aquatic $\dot{V}O_2$ (μmol O_2 g^{-1} h^{-1}) for each individual at each temperature was calculated using the following equation (Fly et al., 2012):

$$\dot{V}O_2 = \frac{\Delta[O_2] \times (V - L)}{\Delta t \times m}$$

Where $\Delta[O_2]$ is the change in water oxygen concentration measured (μmol O_2 L^{-1}), V is the volume of the respiration and L corresponds to the volume of the snail, Δt is the length of time over which the decline in oxygen concentration was observed (h) and m is whole body weight of the animal (g). O_2 measurements were corrected for temperature, salinity, and barometric pressure using the Loligo® Online Oxygen Converter (www.loligosystems.com). Barometric pressure data were obtained from the Plymouth Live Weather Station (http://www.bearsbythesea.co.uk).

Data Analysis

For each experimental temperature, mean snail $\dot{V}O_2$ was calculated and data pooled by population (Figure 1). Preliminary data analysis revealed a relationship between whole body mass and $\dot{V}O_2$ in *L. saxatilis* weighing <0.3g leading to these individuals being excluded from subsequent analyses. A Gaussian function was used to fit thermal performance curves (Angilletta 2006) to each population using MATLAB (Curve Fitting Toolbox™, MathWorks, USA). A

297 Gaussian model was chosen from a selection of plausible models (Gaussian, modified Gaussian,
298 exponentially modified Gaussian, Weibull and quadratic) based on best-fit, evaluated using
299 Akaike Information Criterion (AIC) values (Angilletta 2006).
300
301 Population-specific thermal performance curves were described using four parameters: maximal
302 performance (μ_{max}), optimal temperature (T_{opt}), thermal breadth (T_{br}), and critical temperature
303 limits (CT_{max} and CT_{min}). T_{br} was defined as the temperature range (CT_{min} and CT_{max}) between
304 which respiration rate is within 80% of maximal performance (μ_{max}). Due to a relatively small
305 sample size for each population (n=12), resampling (bootstrap) was used to generate estimates of
306 CT_{min}, CT_{max} (20^{th} and 80^{th} percentile); these values which were then used to estimate thermal
307 breadth (T_{br}). CT_{min} and CT_{max} values were drawn from a normal distribution of values (n = 100)
308 based on the mean and standard deviation values of μ_{max} and T_{opt} derived from the raw data.
309 Coefficients of determination (r^2) associated with the fit of Gaussian curves to population $\dot{V}O_2$
310 data were used to assess the degree of thermal specialism exhibited by each population, where
311 low and high r^2 values indicate thermal generalism and specialism, respectively. Linear mixed-
312 effect (lme) models were used to compare μ_{max} and T_{opt} with the factors: latitude (°N) and
313 species (fixed: *L. littorea; L. saxatilis*), location (random factor), and including an autoregressive
314 (1) autocorrelation term to account for spatial autocorrelation. Linear regression models (linear,
315 exponential and polynomial) were fitted based on lowest AIC scores and tested for significance
316 using ANOVA of the residuals. ANOVA tests were used to compare mean differences in μ_{max}
317 and T_{opt} among locations, with differences among groups identified using Tukey post-hoc tests.
318 All analyses were performed using R (R Core Development Team, 2018).
319
320 **Results**

Maximal performance (μ_{max}) and thermal optima (T_{opt})

There were significant differences in maximal performance (μ_{max}; $F_{1, 113} = 8.08$, $p < 0.01$) among populations depending on latitude and species (Figures 4 & 5). In *L. littorea*, maximal performance decreased at a linear rate of 0.17 μmol O_2 g^{-1} h^{-1} with each degree of latitude ($p < 0.001$). In contrast, in *L. saxatilis* there was a significant non-linear relationship between mean μ_{max} and latitude ($p < 0.001$). Highest maximal performance was recorded in Île de Ré (46.2° N) and Santec (48.7° N) populations (mean performance between sites; 1.94 ± 0.27 μmol O_2 g^{-1} h^{-1}; Tukey pairwise tests, $p > 0.05$), and on average, μ_{max} was 67% lower at Saint-Gilles Croix de Vie (46.4° N), Quiberon (47.3° N) and Plymouth (50.2° N), where mean performance was comparable among sites (mean performance between sites; 1.3 ± 0.20 μmol O_2 g^{-1} h^{-1}; Tukey pairwise tests, $p > 0.05$).

There were also significant differences in thermal optima with latitude depending on the species. Differences were least marked in *L. littorea* where T_{opt} increased with increasing northern latitude (T_{opt}; $F_{1, 113} = 5.34$, $p < 0.05$) from 19.2 to 21.4ºC (11.4% over 4.2º of latitude; Figure 5). In *L. saxatilis*, thermal optima trends were idiosyncratic with generally lower magnitude of change in μ_{max} among locations. There were no significant differences between Quiberon (mean ± SD = 21.9 ± 1.2°C), Santec (mean ± SD = 23.7 ± 1.6°C) and Île de Ré (mean ± SD = 22.9 ± 1.3°C) populations (Tukey pairwise tests, $p > 0.05$). T_{opt} values from the Saint-Gilles Croix de Vie population were ~9% lower than these locations (mean ± SD = 20.8 ± 1.5°C). Thermal optima values for the Plymouth population were significantly lower than those recorded in any of the French populations, but were also highly variable (mean ± SD = 16.8 ± 9.3°C) within the population.

Thermal breadth (Tbr)

346 There were no obvious differences in thermal breadth among populations and between Littorinid
347 species with the exception of *L. saxatilis* in Plymouth, where the thermal breadth was more than
348 double (27.3°C) that of all other locations (12.6 ± 1.3°C) (Figure 5). There was no apparent
349 trade-off between thermal breadth (T_{br}) and maximal performance (μmax) in either species
350 (Figure 6), likely driven by no clear difference in thermal breadth among populations.
351
352 Coefficient of determination (r^2)
353 For both Littorinid species, there was a strong negative relationship between the coefficient of
354 determination (r^2) associated with the fit of a Gaussian curve to population $\dot{V}O_2$ data and latitude.
355 This suggests a shift from thermal specialism in southern populations to thermal generalism in
356 northern populations, especially in *Littorina saxatilis* (Figure 6).
357
358 **Discussion**
359 Patterns of local adaptation along environmental gradients remain poorly characterised in marine
360 taxa (Sanford and Kelly 2011), arguably due to a well-established paradigm that marine species
361 form demographically open populations interconnected by high levels of homogenising gene
362 flow (Grosberg and Cunningham 2001). Using two closely-related congener littorinids with
363 highly contrasting dispersal mechanisms, we assessed the extent to which scale of gene flow
364 (using dispersal capacity as a proxy) influences differentiation in thermal niche traits and
365 emergence of local adaptation. Multiple studies have illustrated that species with contrasting
366 modes of development (i.e. planktonic larvae versus brooding) exhibit different levels of genetic
367 divergence between populations (Hoskin 1997; Watts and Thorpe 2006), including for species of
368 littorinid (Kyle and Boulding 2000; Lee and Boulding 2009). Results revealed marked linear
369 reductions in maximal performance with increasing northerly latitude in the high dispersal-
370 potential *Littorina littorea*; a relationship not observed in the low dispersal-potential *Littorina*

371 *saxatilis*, which appears to show localised specialism of these traits. In *L. littorea*, there appeared
372 to be little evidence of thermal optimisation at different localities. *L. saxatilis*, in contrast,
373 appears to show thermal optimisation, although differences across populations do not follow a
374 simple pattern as would be predicted from latitudinal changes in mean temperatures. Together
375 with limited evidence for changes in thermal breadth (a response predicted to negatively correlate
376 with specialisation), these results suggest physiological plasticity but limited local adaptation in
377 *L. littorea*, whereas in *L. saxatilis*, a combination of plasticity and local adaptation
378 may facilitate persistence.
379
380 Limited evidence of thermal optimisation in *L. littorea* is in line with expectations for species
381 with high levels of interpopulation gene flow, as has been suggested across a range of taxa (e.g.
382 Lenormand, 2002). *Littorina saxatilis* on the other hand, exhibits evidence of local optimisation
383 in a number of traits including μmax and T_{opt}, especially between populations both within France
384 and between France and the UK. The UK population is markedly different from others sampled,
385 with an especially wide thermal breadth in comparison to animals elsewhere, as well as a thermal
386 performance curve that indicates relatively uniform metabolic performance (RMR) across a wide
387 range of temperatures, indicating low thermal sensitivity of metabolism. These differences
388 between the French and UK populations may be indicative of a transition in thermal niche type
389 from thermal specialisms (French populations) to thermal generalism (Plymouth), representing
390 what is arguably a much stronger adaptive divergence between regions than a divergence
391 between values of defined thermal optima.
392
393 The significant differences in thermal optima and thermal breadth of the French populations (and
394 notably Santec) and the UK population (Plymouth) of *L. saxatilis* is unsurprising given (1) the
395 restricted dispersal potential of this species, and (2) barrier to dispersal that the English Channel

396 likely presents. The lack of differentiation between even the most distantly separated French
397 populations is, however, surprising. One explanation may be that interpopulation gene flow
398 between adjacent coastal populations is considerably higher than predicted for this species. The
399 observed pattern could also be explained by a weak underlying selective gradient along the
400 French coast. Indeed, there is evidence that environmental mean plays a limited role in shaping
401 the evolutionary ecology of species relative to other environmental parameters such as variance
402 and predictability (Parker and Begon 1986; Travis 2001). If this is the case, then even very low
403 levels of gene flow could be sufficient for homogenising emergent differences in thermal optima
404 between populations. Alternatively, differences between populations in Plymouth and France
405 may reflect older demographic events, such as these populations originating from different
406 glacial refugia. Doellman et al. (2011) document the complex phylogeographic history of *L.*
407 *saxatilis* in the northeast Atlantic, including evidence for a glacial refuge in the Hurd Deep,
408 which may have been the source of many populations currently occupying southwest England.
409
410 Increasing latitude exhibited strong negative effects on maximal performance (i.e. maximum
411 RMR) of *L. littorea* populations. In contrast, there was differentiation in performance among *L.*
412 *saxatilis* populations, but the relationship was not linear with latitude, as might have been
413 expected in response to linear changes in SST. A negative effect of latitude on μ_{max} in *L. littorea*
414 aligns with specialist-generalist trade-off theory predictions, which forecasts a decline in μ_{max}
415 towards mid-distributional latitudes driven by an adaptive increase in thermal breadth ('a jack of
416 all temperatures is a master of none', Huey and Hertz 1984; Nati et al. 2016). There was,
417 however, no coincidental increase in T_{br} with latitude; a trade-off that might have been expected.
418 Further characterisation of latitudinal patterns in T_{br} across the entire distributional range may
419 reveal a negative association between these two parameters, but the strength and definition of the
420 latitudinal trend in μ_{max} observed here in *L. littorea*, suggests it is unlikely that this pattern could

421 be solely attributed to a negative feedback mechanism driven by the adaptation of T_{br}. To better
422 understand the selective drivers behind this observed trend, investigating how metabolic traits
423 with a more direct link to fitness (e.g. aerobic scope) vary across latitude may shed further light,
424 since a high RMR can be indicative of both compromised and improved performance (i.e. high
425 levels of stress or increased growth and energy assimilation; Thompson and Bayne 1974, Lannig
426 et al. 2010).
427
428 Regardless of the drivers of the trend in μ_{max}, it is surprising to see such strong and fine-scale
429 latitudinal adaptation of this thermal performance curve parameter in *L. littorea*, but no evidence
430 in *L. saxatilis*. Since gene flow cannot easily explain this result, differing selection pressures may
431 be responsible. Although both species were subject to the same environmental temperature
432 regimes, *L. saxatilis* possesses a limited capacity to thermoregulate through behavioural
433 mechanisms (Miller and Denny 2011). There is also evidence that littorinids possess the ability
434 to supress their metabolic rate during periods of temperature stress or aestivation (McMahon
435 1990, 1992; McMahon and Russel-Hunter 1997). Both of these factors could potentially weaken
436 temperature-driven selective pressures acting on maximum RMR in *L. saxatilis*, leading to lesser
437 or no latitudinal adaptation in μ_{max}.
438
439 It is possible that the idiosyncratic nature of the response patterns of μ_{max} and T_{opt} for *L. saxatilis*
440 populations, which exhibit similar polynomial relationships to latitude, is due to interactions
441 between temperature and additional environmental drivers that differed between sites but were
442 not measured. Previous studies have shown that factors including salinity, pH, oxygen, pollution
443 and food availability can affect the thermal tolerance of marine invertebrates over acute and
444 chronic timescales (Denisse Re et al. 2005; Lannig et al. 2006; Pörtner 2010; Schneider et al.
445 2010; Zippay & Hofmann 2010). Whilst it is possible that the idiosyncratic nature of the

response patterns of μmax and Topt for *L. saxatilis* populations are due to interactions between temperature and environmental factors that differed between sites but were not measured we think this is unlikely. All sites were located on the Atlantic seaboard and experience high wave fetch (www.EMODnet-physics.eu) and are therefore well-flushed, are fully marine in terms of salinity (euro-argo.eu), well-oxygenated (>6mg/L, www.oap.ospar.eu), have similar pH (IPCC 2017), and are characterised by high levels of primary production (e.g. Chl-a; www.oap.ospar.eu). Given this, and the overarching importance of environmental temperature in shaping thermal reaction norms in ectotherms (Angilletta, 2006), the non-linear response pattern in *L. saxatilis* populations is most likely due to a mismatch between the latitudinal gradient and the thermal environments of sampling sites rather than the influence of other environmental drivers. One explanation might be geographical variation in the timing of low tide relative to peak daily air temperatures, leading to spatially segregated thermal regimes with variable extremes (Helmuth et al. 2006). *L. saxatilis* could therefore be adapted to a 'thermal mosaic' as opposed to a linear climate-driven thermal gradient (Kuo and Sanford 2009). High-resolution data on spatio-temporal temperature variation at each site would be required to test this hypothesis.

Coefficient of determination (r^2) was used as an additional metric to assess the degree of thermal specialism among populations by providing an indication of how closely the relationship between temperature and metabolism conformed to a typical thermal reaction norm. T_{br} and T_{opt} indicated a shift in thermal specialism between France and the UK in *L. saxatilis* but no change in *L. littorea*. In contrast, r^2 values indicate a transition from thermal specialism to generalism in both species, particularly *L. saxatilis*, suggesting that the thermal niches of both species have undergone significant local adaptation to the latitudinal temperature gradient sampled, perhaps in response to changing temperature variability. In *L. saxatilis*, r^2 reduced to almost zero (1%) in

471 the Plymouth population suggesting a total transition in thermal niche type to thermal generalist.
472 Furthermore, the marked difference between French and UK populations suggests substantial
473 divergence between these populations, mirroring results for T_{opt} and T_{br}, lending further support
474 to the concept that the English Channel acts as a barrier to *L. saxatilis* dispersal, promoting
475 adaptation (and potential remodelling) among populations.
476
477 Taken as a whole, species-specific patterns in population r^2 values in part conformed to
478 expectations based on dispersal capability, and may better illuminate thermal niche adaptation
479 than other metrics (e.g. T_{br} or T_{opt}). The latitudinal trend observed in *L. littorea* could correspond
480 to an amplification of the weak trend in T_{br}. If these trends in r^2 and T_{br} are indeed linked, such
481 results would suggest that *L. littorea* populations are also latitudinally adapted in terms of T_{br} and
482 thermal niche specialism. This in turn would suggest that interpopulation gene flow in this
483 species has thus far been overestimated. A growing body of evidence in a number of taxa has
484 demonstrated a capacity of larvae to use active swimming to vertically migrate, thereby altering
485 exposure to currents of different velocity and limiting dispersal (Knights et al. 2006; Firth et al.
486 2016; Shanks et al. 2009).
487
488 This study supports the growing consensus that local adaptation is more widespread within
489 marine environments than previously assumed with both littorinid congeners exhibiting evidence
490 of adaptive differentiation. The scales and degree of adaptation varied between different thermal
491 niche parameters in both species suggesting a suite of selective pressures of varying strengths is
492 driving differential adaptation among traits. Greater divergence in population T_{opt}, T_{br} and r^2
493 values in the 'low' dispersive *L. saxatilis* relative to the 'highly' dispersive *L. littorea* reinforces
494 the concept that dispersal potential influences the propensity of populations to adapt to localised
495 conditions (Hedgecock 1986; Kawecki and Ebert 2004; Hereford 2009). Yet a strong latitudinal

trend in μ_{max} in *L. littorea* compared to the non-linear relationships of μ_{max} and T_{opt} in *L. saxatilis* might suggest that interpopulation gene flow could be less inhibitive to local adaptation at specific loci than is commonly alluded to in the broader literature. However, as this study only assessed propensity for adaptive differentiation in two species with highly divergent dispersal potentials, these results do not provide a general assessment of the degree to which dispersal influences local adaptation to temperature. Interspecific population differences in thermal performance need to be quantified for a broad range of species which encompass a diversity of taxa and dispersal capabilities. Studies of this nature would also benefit from directly measuring genetic structure across biogeographic gradients.

This study assessed respiration performance under submerged conditions as this is when *Littorina* are most active, and did not consider periods of emersion which can be equally (or more) stressful to organisms (Somero 2002, Helmuth 2006). During emersion, organisms face additional challenges, including desiccation and restricted respiration, both of which can compound physiological stress induced by temperature alone (McMahon 1990). Therefore, intertidal organisms could have a greater thermal tolerance during submersion, meaning the results of this study may underestimate organismal thermal sensitivity and degree of differentiation between populations (Stenseng et al. 2005). If this is the case, then the fact that differences in population performance were still observed under submerged conditions for *L. saxatilis* provides strong evidence for local adaptation to temperature in this species. In order to speculate on the effects of rising temperatures for the future performance of the study species, thermal tolerance would need to be assessed in air using suitable physiological parameters (e.g. mitochondrial respiration, membrane fluidity or nerve function, see Stenseng et al. (2005)).

520 The body temperature of intertidal invertebrates can deviate substantially from environmental
521 temperatures in air (Helmuth et al. 2006, 2011). Direct exposure to high levels of solar radiation
522 can rapidly raise the body temperature of organisms by several degrees above ambient.
523 Conversely, wave splash and convective cooling can counteract the effects of significant
524 increases in air temperature (Helmuth et al. 2011). The degree of decoupling between body
525 temperature and environmental temperature can also vary with organism size, morphology,
526 colour and behaviour (Miller and Denny, 2011; Nicastro et al. 2012), which may further
527 influence responses. Though not the focus of this study (body temperature closely traces
528 environmental temperature in water (Helmuth 1999)), it should be an important consideration
529 when aiming to understand climate change impacts on intertidal communities. It is important that
530 future studies (where possible) utilise novel methods to record high resolution data on body
531 temperature and environmental temperature simultaneously (see Helmuth et al. 2016). This will
532 enable us to better understand the multi-dimensional temperature-driven selective environment
533 which is shaping the thermal responses of intertidal ectotherms.
534
535 Environmental filtering preceding recruitment can result in genetically and phenotypically
536 distinct populations with optimised fitness in their local environments independent of
537 evolutionary processes (Schmidt and Rand, 2001). While evidence of local adaptation in
538 performance was found, patterns did not fully follow those expected under thermal niche
539 differentiation models. Non-genetic processes such as developmental plasticity, phenotypic
540 plasticity, maternal effects and transgenerational acclimation may also act to 'fine-tune'
541 populations to their local environments (Kawecki and Ebert 2004; Marshall 2008); a process
542 which can be difficult or impossible to reverse (Kinne 1962; Zamer and Mangum 1979). As
543 such, there is a need to separate the environmental, phenotypic and genetic components of niche
544 differentiation in order to understand the dynamics and extent of local adaptation in marine

545 species, although it is likely that a combination of plasticity and adaptation are key determinants
546 of species performance, distributions and persistence under climate change (Somero 2010;
547 Valladares et al. 2014). Local adaptation research must therefore position itself among the
548 priorities of future climate change research if we are to better understand and predict species-
549 level responses to climate change.
550

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557

558 **References**

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Figures

Figure 1 | (Top) Distribution of *Littorina littorea* and *Littorina saxatilis* along the coastlines of the North East Atlantic, and (Bottom) collection locations for populations of *L. littorea* and *L. saxatilis*. Top: Points denote reported occurrences catalogued within the Global Biodiversity Information Facility (GBIF) and Ocean Biogeographic Information System (OBIS) databases. Semi-transparent lines indicate the complete distribution of each species as estimated by Johannesson (1988). *Littorina saxatilis* is documented as an alien species in the Mediterranean Sea but is shown here for completeness (Streftaris et al. 2005).

Figure 2 | A range of thermal performance curves (TPCs) quantifying an organisms' 'thermal niche' trait performance over a range of temperatures as expected for locally-adapted populations distributed along a latitudinal temperature gradient in the northern hemisphere. Based on optimality models and specialist-generalist trade-offs (Levins, 1968; Mitchell and Valone 1990), intraspecific variation in TPCs is predicted to be characterised by i) a decrease in T_{opt} with increasing latitude, ii) an increase in T_{br} with increasing latitude (due to greater seasonal variability), and iii) a compensatory decline in μ_{max} with increasing latitude (i.e. performance trade-off with T_{br}). NB: Polar regions are characterised by highly stenothermal conditions and predicted to reduce T_{br} and increase in μ_{max}. Inset shows key TPC features: the temperature at which performance is maximised (thermal optimum, or T_{opt}); maximal performance in the measured trait (μ_{max}); the temperature range over which performance is maximised (thermal breadth, or T_{br}) and; critical temperature limits beyond which performance is compromised (CT_{min} and CT_{max}). Here, T_{br} is defined as the temperature range in which performance was within 1.28 standard deviations of T_{opt} (i.e. between the 20^{th} and 80^{th} percentile)**.**

Figure 3 | Trends in Sea Surface Temperature (SST) with latitude. Data shown are average monthly mean SST (°C) for the five-year period 2011-2015. SST data were obtained from the Earth System Research Laboratory: National Oceanic and Atmospheric Administration (NOAA Optimum Interpolation Sea Surface Temperature; www.esrl.noaa.gov). Significant regression (± 95% Confidence Limits) is shown (Mean temperature (°C) = 39.6 – 0.58·Northing; $R^2 = 0.03$).

Figure 4 | Thermal sensitivity of metabolism in five populations of *Littorina littorea* and *Littorina saxatilis*. Thermal performance curves for populations based on metabolic rate ($\dot{V}O_2$ as proxy). Grey points represent mean oxygen consumption per individual snail at the given temperature.

Figure 5 | a-b: Maximal performance (μ_{max}) and, **c-d:** thermal optima (T_{opt} - °C) of individual snails collected across a latitudinal gradient (n=12 per location). **e-f**: Bootstrap estimates (n=100 per locations) of mean thermal optima (ºC; closed circles), minimum and maximum critical temperatures (indicated by lower and upper tips of error bars respectively; °C), and thermal breadth (ºC; $T_{br} = CT_{max} - CT_{min}$; open circles) of Littorinids across a latitudinal gradient. Significant regressions (-) are shown ($p < 0.05$) based on *lme* model outputs (see body text; not fitted for CT values). Regressions: (a) µmax = $8.98 – 0.158x$, $R^2 = 0.71$; (b) µmax = $18800 – 1170x + 24.4x^2 - 0.169x^3$, $R^2 = 0.57$; (c) Topt = $26x – 0.26x^2 – 610$, $R^2 = 0.43$; (d) Topt = $91400 – 5720x + 119x^2 - 0.83x^3$, $R^2 = 0.16$; (e) Thermal breadth – *L. saxatilis* = $1.2+\exp^{1.1E\text{-}21x}$; $R^2 = 0.36$).

Figure 6 | Top: Relationship between thermal breadth (°C) and maximal performance (µmax ± SD) and; **Bottom:** The coefficient of determination (r^2) associated with model fits to $\dot{V}O_2$ data from five locations across a latitudinal temperature gradient. Significant regressions are shown:

926 *Littorina littorea* ($CoD_{litt} = 3.21 - 0.06 \cdot latitude$; $R^2 = 0.64$); *Littorina saxatilis* ($CoD_{sax} = 5.40 -$

927 $0.11 \cdot latitude$; $R^2 = 0.78$).

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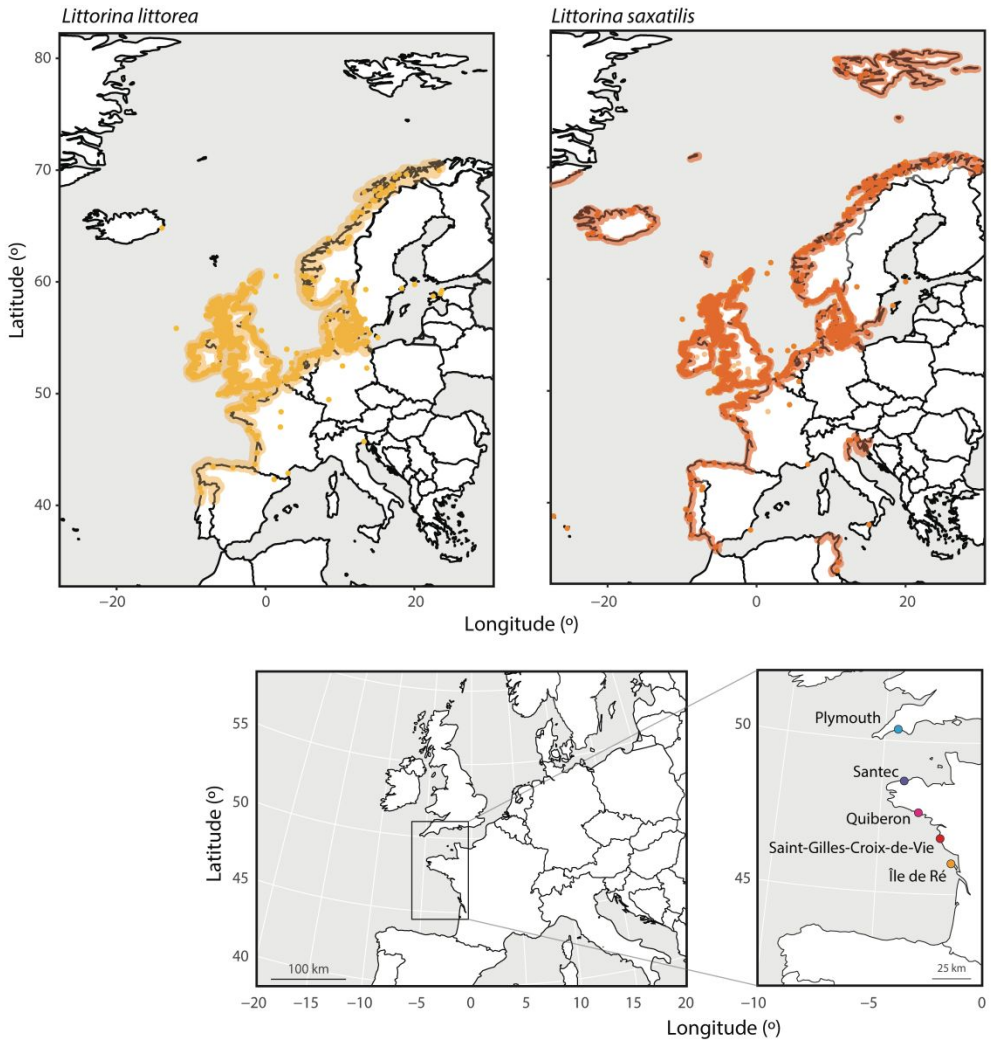

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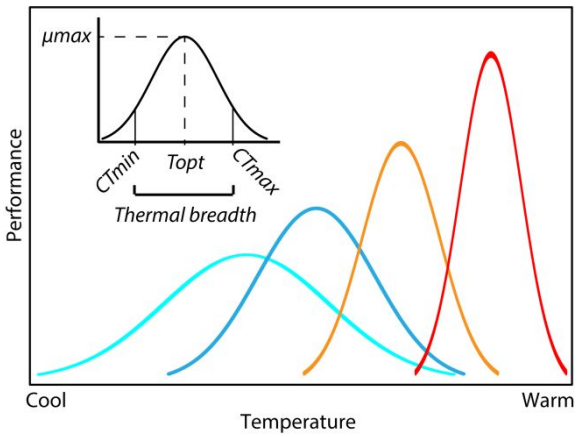
μmax
CTmin
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Performance
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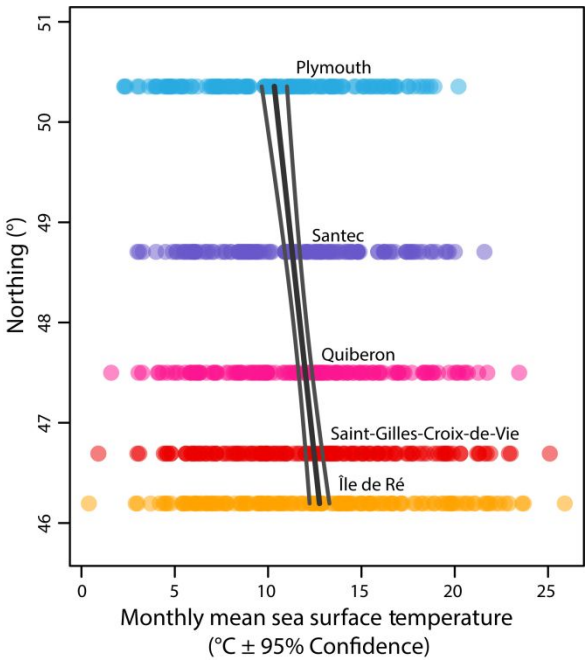
Plymouth
Santec
Quiberon
Saint-Gilles-Croix-de-Vie
Île de Ré
Northing (°)
51
50
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15
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25
Monthly mean sea surface temperature
(°C ± 95% Confidence)

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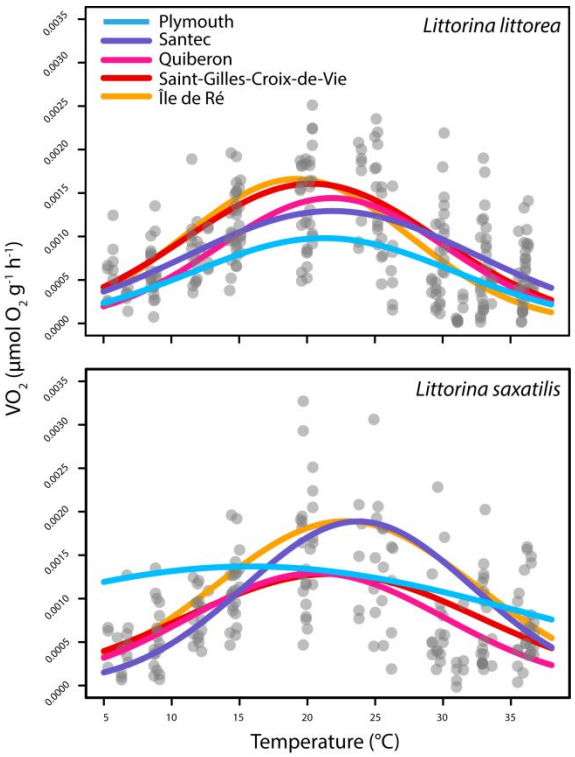

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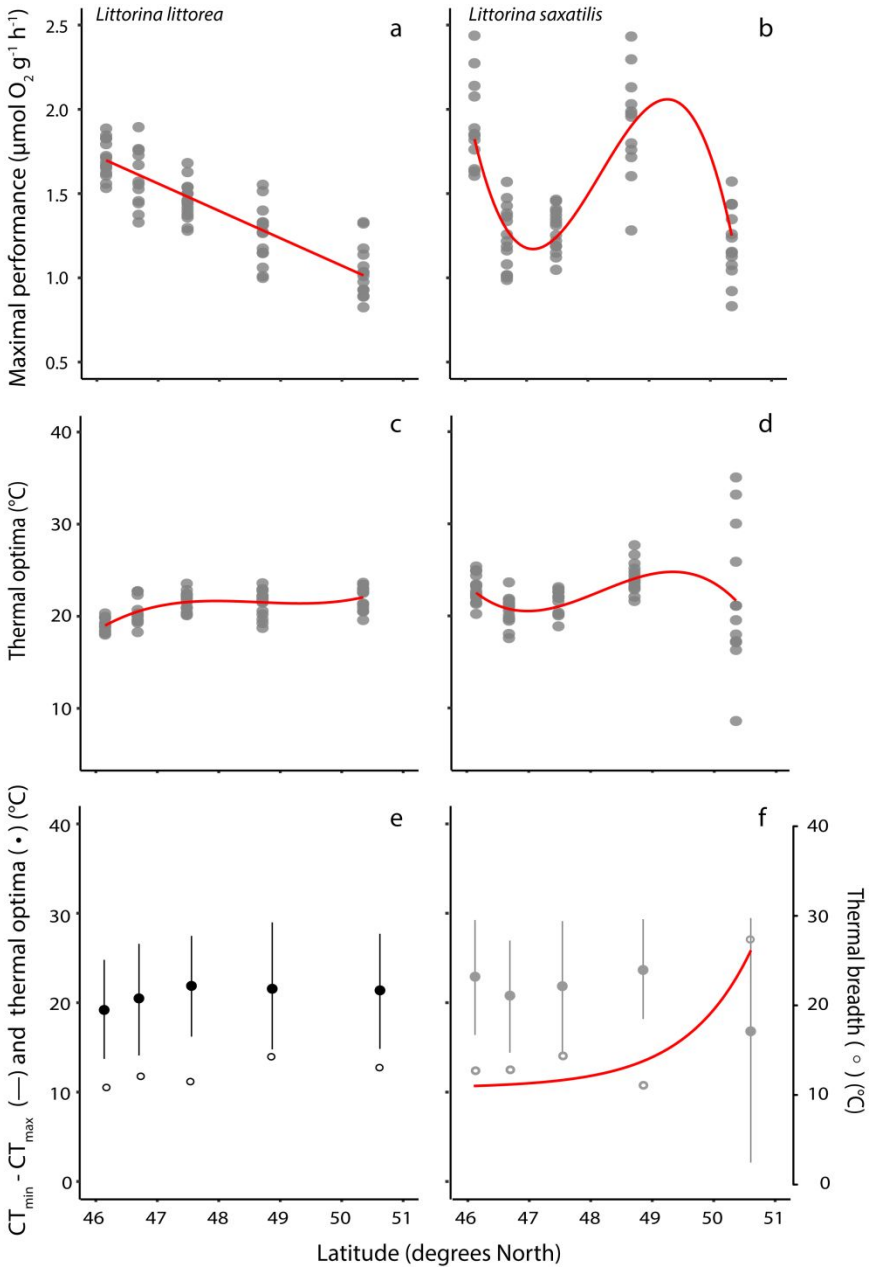
Littorina littorea
Littorina saxatilis
a
b
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d
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Maximal performance (µmol O_2 g^{-1} h^{-1})
Thermal optima (°C)
CT_{min} - CT_{max} (—) and thermal optima (•) (°C)
Thermal breadth (○) (°C)
Latitude (degrees North)

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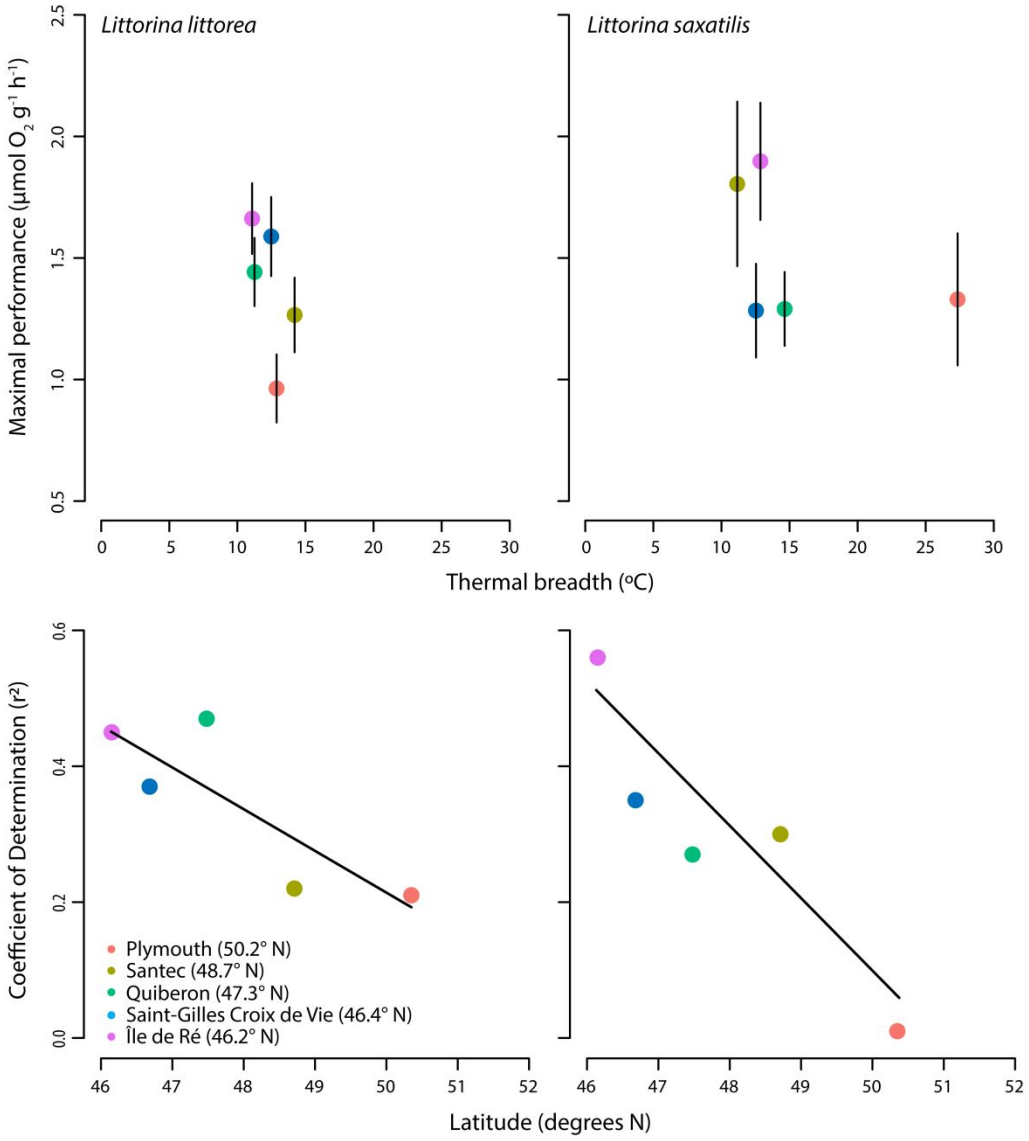
Littorina littorea
Littorina saxatilis
Maximal performance (µmol O_2 g^{-1} h^{-1})
Thermal breadth (ºC)
Coefficient of Determination (r^2)
Latitude (degrees N)
Plymouth (50.2° N)
Santec (48.7° N)
Quiberon (47.3° N)
Saint-Gilles Croix de Vie (46.4° N)
Île de Ré (46.2° N)

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